Expression of Interleukin-4 in the Epidermis of Transgenic Mice Results in a Pruritic Inflammatory Skin Disease: An Experimental Animal Model to Study Atopic Dermatitis

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Atopic dermatitis, a common, chronic, inflammatory skin disease that occurs with increasing prevalence, is characterized by hyperactivated cytokines of helper T cell subset 2 and is frequently associated with staphylococcal infection. An experimental animal model of atopic dermatitis induced by transgenically introduced cytokine is not available. We generated a transgenic mouse line expressing epidermal interleukin-4, a critical cytokine of helper T cell subset 2. Here we show that transgenic mice spontaneously developed a pruritic inflammatory skin disease reproducing all key features of human atopic dermatitis, including xerosis, conjunctivitis, inflammatory skin lesions, *Staphylococcus aureus* infection, histopathology of chronic dermatitis with T cell, mast cell, macrophage-like mononuclear cell, and eosinophil infiltration, and elevation of total serum IgE and IgG1. The onset and early progression of skin disease coincided with increased total serum IgE and IgG1. The mouse disease occurred at a 43% annual incidence rate and primarily affected the poorly haired skin: ear (100%), neck (65%), eye (53%), face (29%), tail (12%), leg (12%), and torso (6%). As a group the affected transgenic mice manifested with a skin disorder that fulfilled the clinical diagnostic criteria established for atopic dermatitis in human patients. Pending further characterization to authenticate it as a model of atopic dermatitis, this experimental animal model of pruritic inflammatory skin disease may facilitate investigations for the roles of interleukin-4 in cutaneous inflammation and skin infection in human patients. ***Key words: IgE/mast cell/eosinophil/T cell/staphylococcus. J Invest Dermatol 117:977–983, 2001***

Atopic dermatitis (AD) is a common, chronic, inflammatory, pruritic (itchy) skin disease that affects a large population of people and is the most common skin disease affecting children younger than 11 y of age (Hanifin and Rajka, 1980; Leung *et al*, 1999). The onset of AD is usually in childhood, but 70% of severe cases of AD will carry forward to adulthood (Arikian *et al*, 1998). Although not life-threatening, AD has significant impact on patients' quality of life and the national economy, with the national medical costs for AD care estimated to be 360 million dollars in 1990 (Arikian *et al*, 1998; O'Hare and Krowchuk, 1998). Over the last four decades, AD has occurred with increasing prevalence among school children in the developed countries, from about 7% in the 1970s to 20% in the 1990s (Leung *et al*, 1999).

The definite etiology of AD is not yet determined and multiple pathogenic factors have been implicated. These factors include hyperactivation of T helper cell subset 2 (Th2) immune response (Paul and Seder, 1994; Leung *et al*, 1999), autoimmunity (Valenta *et al*, 1998), infection (Arikian *et al*, 1998; Hofer *et al*, 1999; Leung *et al*, 1999), allergens (Mudde *et al*, 1990; Leung *et al*, 1999), and genetics (Kawashima *et al*, 1998; Leung *et al*, 1999). The most recent work has focused on the immune etiology. Furthermore, controversies still exist regarding the role of the Th2 and Th1 immune system in the pathogenesis of AD, particularly in the chronic phase of the disease (Grewe *et al*, 1994; Hamid *et al*, 1994). An experimental animal model of AD induced by transgenically introduced Th-subset-specific cytokine, if available, would facilitate investigations of the relationship between cytokines and skin infection, and the step-by-step immunologic sequences of events before and at disease onset and during disease progression.

Because of the need for such an experimental animal model of AD, we explore whether an epidermally expressed Th2 cytokine will affect the cutaneous immune system in such a way that an AD disease clinical phenotype, along with its associated infection, can be induced. In particular, we ask the question whether epidermal expression of interleukin-4 (IL-4), the critical Th2 cytokine (Paul and Seder, 1994), can induce a clinical phenotype of AD and to what extent the experimental model recapitulates key features of human AD. The workability of this approach is supported by reports that epidermally expressed cytokines alter skin immunology (Turksen *et al*, 1992; Carroll *et al*, 1997). Towards that end, transgenic (Tg) mice were produced containing the murine IL-4 gene at the basal keratinocytes. Here we report that Tg mice expressing IL-4 in the skin developed a chronic, inflammatory, pruritic skin disease associated with bacterial infection and that the mouse disease phenotype is clinically, microbiologically, pathologically, and serologically identical to AD in humans and fulfills

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Abbreviations: AD, atopic dermatitis; Tg, transgenic; Th2, T helper cell subset 2.

the clinical diagnostic criteria for AD in human patients. With further characterization to authenticate it as a model of AD, this experimental mouse model of pruritic inflammatory skin disease may be helpful for gaining insight on the roles of Th2 cytokines in cutaneous inflammation and in the skin immune defense against infection.

MATERIALS AND METHODS

Tissue-specific IL-4 Tg vector construction A cDNA fragment containing the entire IL-4 coding region flanked by BamH I/Bgl II sites was generated by polymerase chain reaction (PCR) (Xu *et al*, 1998) with a mouse IL-4 cDNA template (American Type Culture Collection, Rockville, MD) and the following primer pairs: forward primer 5′-GGATCCGGATCCCAGAGCTATTGATGGGTCTC-3′ and reverse primer 5′-AGATCTAGATCTGCATGGTGGCTCAGTACTAC-3′. The PCR product contains the murine IL-4 sequence corresponding to nucleotide 45-494, including portions of 5′- and 3′-untranslated regions, start and stop codons, and the entire coding region (Lee *et al*, 1986). The PCR product was ligated into a PCR cloning vector (PCR-II, Invitrogen, San Diego, CA). The following BamH I/Bgl II digestion yielded the desired fragment with sticky ends. This fragment was subcloned into BamH-I-linearized pG3Z-K14 vector (originally constructed and modified by Dr. Elaine Fuchs, Howard Hughes Medical Institute, University of Chicago) to produce a pK14–IL-4 construct (Vassar *et al*, 1989). This construct contains basal keratinocyte (keratin 14) specific promoter/enhancer, rabbit β-globin intron, murine IL-4 cDNA, and K14 polyA tail (**Fig 1**).

Generation of IL-4 Tg mouse line The pK14–IL-4 Tg construct was linearized by EcoR I/Hind III digestion, gel purified, and then sent to Jackson Laboratory (Bar Harbor, ME) for microinjection into oocytes of CByB6 F2 mice. The injected oocytes were implanted in CByB6 F1 pseudopregnant mice. The offspring born from these mice were examined for the incorporation of IL-4 by southern blot analyses using genomic DNA extracted from tail clippings and murine IL-4 cDNA as a probe (Jackson Laboratory). The positive IL-4 Tg mice, confirmed by a repeated southern blot analysis, were then mated with non-Tg BALB/cBy mice (Jackson Laboratory). The mice were housed in conventional cages, along with their non-Tg littermates, fed with standard mouse chow and water, and observed without any manipulation.

Analysis of tissue-specific IL-4 mRNA expression Biopsies obtained from normal skin of the IL-4 Tg mice and non-Tg littermates were immediately placed in RNAlater solution (Ambion, Austin, TX), stored at 4°C and used within 1 mo. The skin was homogenized in RNAzol B solution (Tel-Test, Friendswood, TX) and the total RNA was extracted according to the manufacturer's protocol. DNA contamination was removed by treating the RNA in RNase-free DNase I at 37°C for 10 min (5 U per μg RNA; Pharmacia, Milwaukee, WI). The presence of IL-4 mRNA was examined by a 35-cycled reverse transcriptase PCR (RT-PCR) (Xu *et al*, 1998) using 1 μg total RNA with reverse primer 5′-CAGTGATGTGGACTTGGACTCATTCAT-GGTGC-3′ and forward primer 5′-CCAGCTAGTTGTCATCCTGC-TCTTCTTTCTCG-3′ (Clontech, Palo Alto, CA) and an RT-PCR kit (Gibco-BRL, Grand Island, NY). This primer pair amplifies a 357 bp PCR product corresponding to nucleotide 70-427 (Lee *et al*, 1986). β-actin mRNA as a housekeeping gene control was similarly examined using the following primer pairs: reverse primer 5′-CTCCTTAATGT-CACGCACGATTTC-3′ and forward primer 5′-GTGGGGCGCCCC-AGGCACCA-3′ (**Fig 1**). To confirm the nucleotide sequence of the RT-PCR-amplified IL-4 cDNA fragment, the sequence of the IL-4 fragment flanked by the above primer pair was analyzed by a MacVector program for restriction sites (Oxford Molecular, Campbell, CA). Pst I (Pharmacia, Milwaukee, WI) and EcoR I (Gibco-BRL) were used as enzymes that would positively and negatively, respectively, digest the fragment. The IL-4 cDNA control (Clontech) and λ DNA (Pharmacia) were included as positive DNA controls for the enzymes Pst I and EcoR I, respectively.

Clinical observation The skin of Tg and non-Tg mice was examined for texture and inflammatory lesions and photographed. The skin disease severity scores were recorded according to a modified method (Leung *et al*, 1990; Matsuda *et al*, 1997). Pus was obtained in areas of severe cutaneous infection (all from external ears) for bacterial culture.

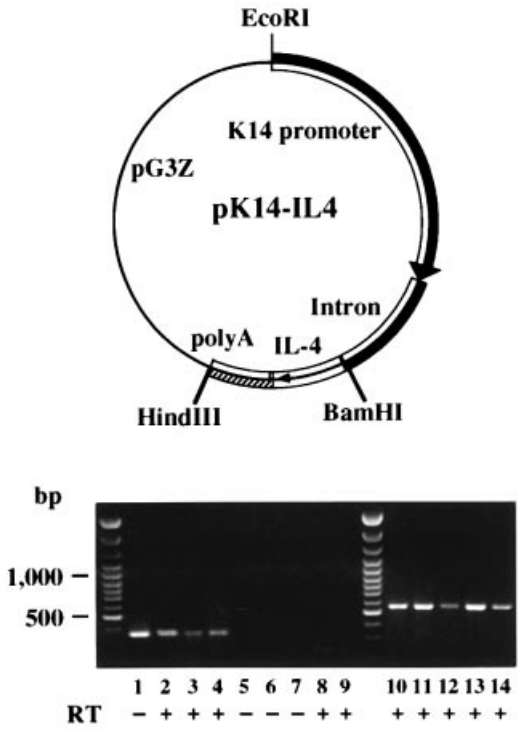

Figure 1. The pK14–-IL-4 Tg construct and the skin mRNA expression of the IL-4 Tg mice. The complete pK14–IL-4 Tg construct contains the PG3Z vector, the K14 promoter, the rabbit β-globin intron, the murine IL-4 cDNA, and a K14 polyA tail (*upper panel*). The skin mRNA expression of the IL-4 Tg mice in comparison with the non-Tg littermates (*lower panel*). Total RNA was extracted from normal skin. RT-PCR was performed with murine IL-4-specific primer pair on total RNA extracted from three Tg mice (*lanes 2–7*) and two non-Tg littermates (*lanes 8, 9*). RT-PCR was performed with the mouse skin RNA in the presence (*lanes 2–4*, *8*, *9*) and absence (*lanes 5–7*) of reverse transcriptase. RT-PCR was also performed with β-actin primer pair on the same amount of RNA from the same Tg mice (*lanes 10–12*) and the same non-Tg littermates (*lanes 13*, *14*). *Lane 1* is the PCR product performed with the same IL-4 primer pair on a murine IL-4 cDNA control provided by Clontech. The molecular size standards included are 100 bp DNA ladders.

Histopathology Biopsies obtained from normal skin of the IL-4 Tg mice and non-Tg littermates and skin lesions of the IL-4 Tg mice were fixed in formalin, embedded in paraffin, and processed for hematoxylin–eosin, periodic acid–Schiff, Giemsa, and Gramís staining. To quantify the relative number of inflammatory cells, the histologic sections of normal skin of non-Tg mice and early and late inflammatory skin lesions of affected Tg mice were examined under a high power field (50× objective lens) and the number of inflammatory cells were counted and averaged (five fields per histology section, five mice in each of the three groups). Statistical analyses of the inflammatory cell types, mononuclear cells, mast cells, and eosinophils were performed with Internet software VassarStats at the following website: http://vassar.edu.

Immunopathology Biopsies were obtained from normal skin of the IL-4 Tg mice and non-Tg littermates and skin lesions of the IL-4 Tg mice and immediately frozen in OCT compound. Cryosections were sequentially incubated with rat monoclonal antibody to mouse CD3 (Serotec, Raleigh, NC), followed by fluorescein isothiocyanate labeled goat antirat IgG (Kirkeggard and Perry, Gaithersburg, MD). Cryosections were also incubated with biotin-labeled monoclonal antimouse CD4 or CD8 (Serotec), followed by streptavidin-labeled Texas Red (Kirkeggard and Perry).

Enzyme-linked immunosorbent assay (ELISA) For total serum IgE levels, microtiter plates were coated with monoclonal rat antimouse IgE (2 μg per ml, Pharmingen, San Diego, CA), followed by sequential incubation of serially diluted purified mouse IgE (Pharmingen) or sera (in duplicate) from the IL-4 Tg and non-Tg mice, biotinylated rat

monoclonal antimouse IgE (2 μg per ml, Pharmingen), streptavidin alkaline phosphatase (1:1000, Pharmingen), and then an alkaline phosphatase substrate (Bio-Rad, Hercule, CA). Optical density was measured as absorbance in an Optimax microplate reader at 405 nm (Molecular Devices, Sunnyvale, CA) (**Fig 4**). Total serum IgE levels were calculated using the standard curve generated from the results obtained with the purified mouse IgE. Similarly, microtiter plates were coated with monoclonal rat antimouse IgG1 or IgG2a (1:2000 dilution; Serotec), followed by sequential incubation of serially diluted purified mouse IgG1 or IgG2a (Sigma, St. Louis, MO) or sera (in duplicate) from the Tg and non-Tg mice, alkaline-phosphatase-labeled rat antimouse IgG1 or IgG2a (1:500 dilution, Zymed, South San Francisco, CA), and then the substrate. Measurement of optical density and calculation of concentrations were performed as above. For serum IL-4 level, sera from affected Tg mice and non-Tg littermates were similarly assayed with a commercially available mouse IL-4 ELISA kit sensitive to 7.8 pg per ml (the lowest mouse IL-4 standard in the assay recommended by the manufacturer, R&D Systems, Minneapolis, MN). The expected value of normal mouse serum IL-4 according to a large number of samples previously tested is zero (or undetected) ($N = 40$, Cat. No. M4000, R&D Systems).

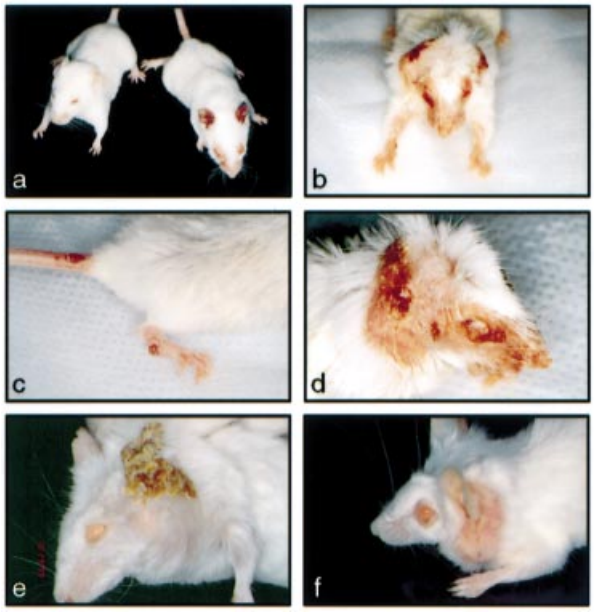

Figure 2. The affected IL-4 Tg mice demonstrate inflammatory skin disease clinically. At the age of 4 mo, a male Tg mouse (*a*, *right side*) developed an inflammatory skin lesion clearly visible on the ears, mouth, and around the eyes. A male non-Tg littermate (*a*, *left side*) is pictured for comparison. At the age of 8 mo, the disease progressed in the same Tg mouse (*b–d*).There was severe dermatitis of the head and neck with areas of crusting, hair loss, and bacterial pyoderma (*d*) as well as destruction of external ears and right eye blindness from corneal and conjunctival scarring (*b*, frontal view; *d*, right lateral view). The tail and the right hind leg showed dry skin and excoriation (*c*). Severe bacterial pyoderma was illustrated in two other affected mice (*e*, *f*). Bacterial culture documented the growth of *Staphylococcus aureus* (*d*, *e*, *f*).

RESULTS

Generation of Tg mice The strategy for the IL-4 Tg construct is detailed in **Fig 1** (*upper panel*). The IL-4 gene incorporation successfully occurred in two founder mice (CByB6 strain), one male and one female, confirmed by repeated southern blot analyses. The founders ($N = 2$) were mated with non-Tg BALB/cBy strain mice to produce 38 offspring.

The skin expression of IL-4 transgene The skin-specific expression of IL-4 mRNA in Tg mice was determined by RT-PCR performed on total RNA extracted from normal skin obtained from the Tg and non-Tg mice. RT-PCR demonstrated the presence and absence of IL-4 mRNA (a 357 bp product) in the Tg mice and in the non-Tg littermates, respectively (**Fig 1**, *lower panel*). This 357 bp RT-PCR product comigrated with a PCR product flanked by the same primer pair on a control mouse IL-4 cDNA template. The omission of reverse transcriptase in the reaction solution totally eliminated the IL-4 expression, ruling out DNA template contamination (**Fig 1**). The control β-actin cDNA was present in equal amounts in the Tg and non-Tg mice total RNA, confirming the equal quality and quantity of mRNA present in total RNA extracted from Tg and non-Tg mouse skin. Pst I completely digested the 357 bp IL-4 PCR fragments generated

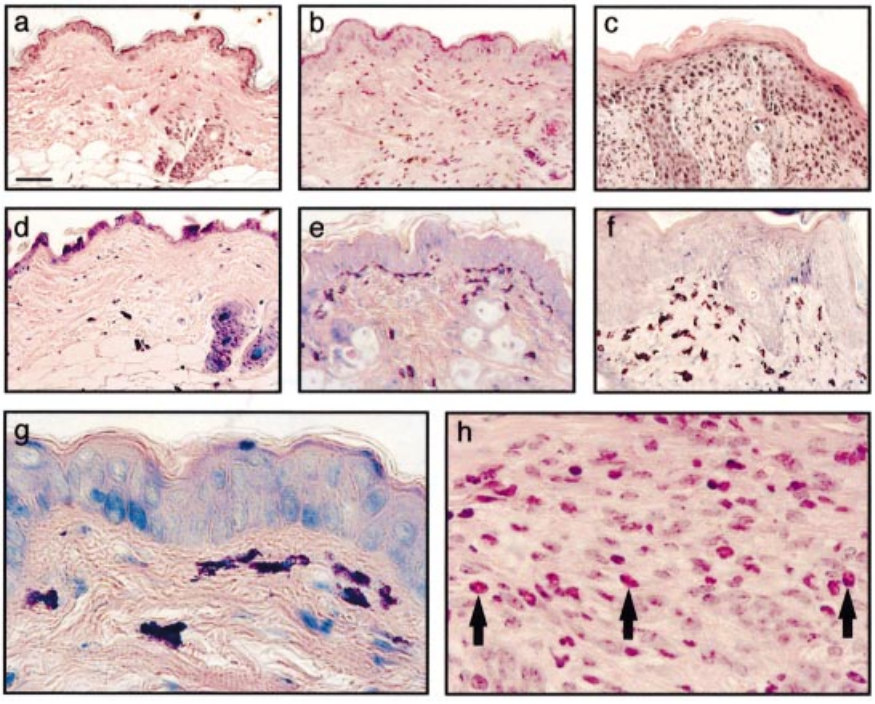

Figure 3. The pathology of affected IL-4 Tg mice showed dermatitis with infiltration of mononuclear cells, mast cells, and eosinophils. Skin biopsies from a non-Tg littermate (*a*, *d*) and early skin lesions (*b*, *e*, *g*) and chronic lesions (*c*, *f*, *h*) of IL-4 Tg mice were fixed in formalin, embedded in paraffin, and processed for hematoxylin–eosin (*a–c*, *h*) and Giemsa (*d–g*)staining. Arrows indicate eosinophils (*h*).*Scale bar*: 50 μm (*a–f*), 10 μm (*g*, *h*).

Table I. Skin lesions distributed primarily in hairless areas

Mice	Ear	Neck	Eye	Face	Tail	Leg	Torso	Pyoderma
1	+	+	+	–	–	–	–	–
2	+	+	+	–	–	–	–	+
3	+	+	–	+	+	–	+	+
4	+	+	–	–	–	–	–	+
5	+	+	–	–	–	–	–	–
6	+	–	–	–	–	–	–	–
7	+	–	+	–	–	–	–	–
8	+	+	+	+	+	+	–	+
9	+	+	–	–	–	–	–	–
10	+	+	–	–	–	–	–	+
11	+	+	+	+	–	–	–	+
12	+	–	+	–	–	–	–	–
13	+	–	+	–	–	–	–	–
14	+	–	+	+	–	+	–	–
15	+	+	–	–	–	–	–	+
16	+	–	+	+	–	–	–	–
17	+	+	–	–	–	–	–	+
total percentage	100	65	53	29	12	12	6	47

from both the IL-4 cDNA template control and the total RNA of the Tg mice into two fragments of expected sizes (76 bp and 281 bp), confirming the nucleotide sequence of the RT-PCR-amplified IL-4 cDNA fragment. EcoRI digested λ DNA, but did not digest the mouse IL-4 cDNA fragment as predicted (data not shown).

Tg mice developed a chronic inflammatory skin disorder and skin infection The skin of the newborn Tg mice was not affected. At the age of 4 mo, xerosis (dry skin) and inflammatory skin lesions surfaced and the skin lesions appeared to be pruritic (as evidenced by the constant face rubbing and scratch motions of the affected mice and the absence of such behavior in the unaffected mice) (**Fig 2*a***). The skin lesions initially occurred at the mice's ears and subsequently extended to the neck, mouth, around the eyes, tail, and legs. Eyelid dermatitis, blepharitis, and conjunctivitis resulted in corneal and conjunctival scarring (**Fig 2*b***, ***d***). Self-inflicted excoriation followed by infection in the skin lesions (as shown clinically by pustules and crusting) slowly destroyed the external ears (**Fig 2*b***, ***d***, ***e***). Among the total 40 mice (founders and their offspring), 17 developed dermatitis within a 12 mo observation period (from birth to 1 y of age), with an annual incidence of 43%. None of the non-Tg littermates developed skin lesions during this period ($N = 9$). The affected mice included the female founder and seven of her offspring (six male, one female) and nine offspring of the male founder (five male, four female). The lesions predominantly affected poorly haired skin: ear (100%), neck (65%), eye (53%), face (29%), tail (12%), leg (12%), and torso (6%) (**Table I**). This distribution is similar to the affected skin location in canine AD (Willemse, 1986) and a spontaneously arising AD-like skin lesion in an NC/Nga mouse strain (Matsuda *et al*, 1997). Among the 17 Tg mice affected with skin inflammation, eight (47%) developed bacterial pyoderma at the external ear areas; none of the non-Tg littermates or the Tg mice unaffected with skin inflammation developed pyoderma (**Fig 2*d–f***) (**Table I**). Inflammatory skin lesions preceded the onset of pyoderma in all cases. Microbiologically, bacterial cultures documented the presence of *Staphylococcus aureus* (eight of eight mice) and *Pseudomonas aeruginosa* (seven of eight mice) in the pus obtained from mice affected with bacterial pyoderma. The staphylococcal infection has been documented to be a common infectious complication and exacerbating factor in human patients with AD (Hofer *et al*, 1999; Leung *et al*, 1999). Pseudomonas otitis externa is commonly observed in canine patients affected with AD (Dr. Thierry Olivry, North Carolina State University College of Veterinary Medicine, unpublished observation). These skin manifestations in our IL-4 Tg mice resemble the typical presentation in human patients with AD (Hanifin and Rajka, 1980; Leung *et al*, 1999).

The pathology of the Tg mice skin lesions is characterized by chronic dermatitis, mononuclear cell infiltration, and mast cell degranulation In the non-Tg littermates histologic examination of their skin revealed normal epidermal thickness and trace mononuclear cells in the dermis (**Fig 3*a***). Histologic examination of the normal appearing skin from the Tg mice revealed essentially no pathology (data not shown). In the early inflammatory lesions, the Tg mouse skin demonstrated mild spongiosis and acanthosis, and a dermal and epidermal infiltration of mononuclear cells (**Fig 3*b***). Actively degranulating mast cells in the dermis, the number ranging from few to moderate to numerous, were observed by Giemsa staining (**Fig 3*e***, ***g***). In the chronic inflammatory lesions, the Tg mouse skin demonstrated features of chronic dermatitis, including acanthosis of the epidermis with mild spongiosis, hyperkeratosis with focal areas of parakeratosis, and a prominent dermal mononuclear cell infiltration (**Fig 3*c***). A number of nondegranulating and minimally degranulating mast cells were observed in the dermis by Giemsa staining, ranging from few to numerous (**Fig 3*f***). A moderate degree of eosinophil infiltration was also observed in the dermis of chronic lesions (**Fig 3*h***). A few macrophage-like mononuclear cells were identified in the dermis of early and chronic skin lesions. In crusting skin lesions, epidermis was absent and was replaced by serous materials and numerous neutrophils. Gram+ cocci in clusters and Gram bacilli were detected. Periodic acid–Schiff stain detected no fungal elements (data not shown). These pathologic findings resemble those observed in chronic AD lesions of human patients (Mihm *et al*, 1976). The mean number of inflammatory cells (per high power field, 50×) in the non-Tg mouse skin, early Tg mouse lesion, and late Tg mouse lesion, respectively, were calculated as the following: mononuclear cells, 34 (±8.0, SD), 94 (±8.6 SD), 138 (±56.5 SD); mast cells, 4 (±1.6 SD), 22 (±5.8 SD), 39 (±10.4 SD); eosinophils, 0, 0, 8 (±4.3 SD). The p values calculated by one-tailed *t* test (independent sample formula) for comparing the mean number of inflammatory cells between non-Tg mouse skin and early Tg mouse lesion, between non-Tg mouse skin and late Tg mouse lesion, and between early and late Tg mouse lesions, respectively, were as follows: mononuclear cells, <0.0001, <0.0001, <0.0002; mast cells, <0.0001, <0.0001, <0.0001; eosinophils, not applicable, <0.0001, <0.0001 (SD, standard deviation).

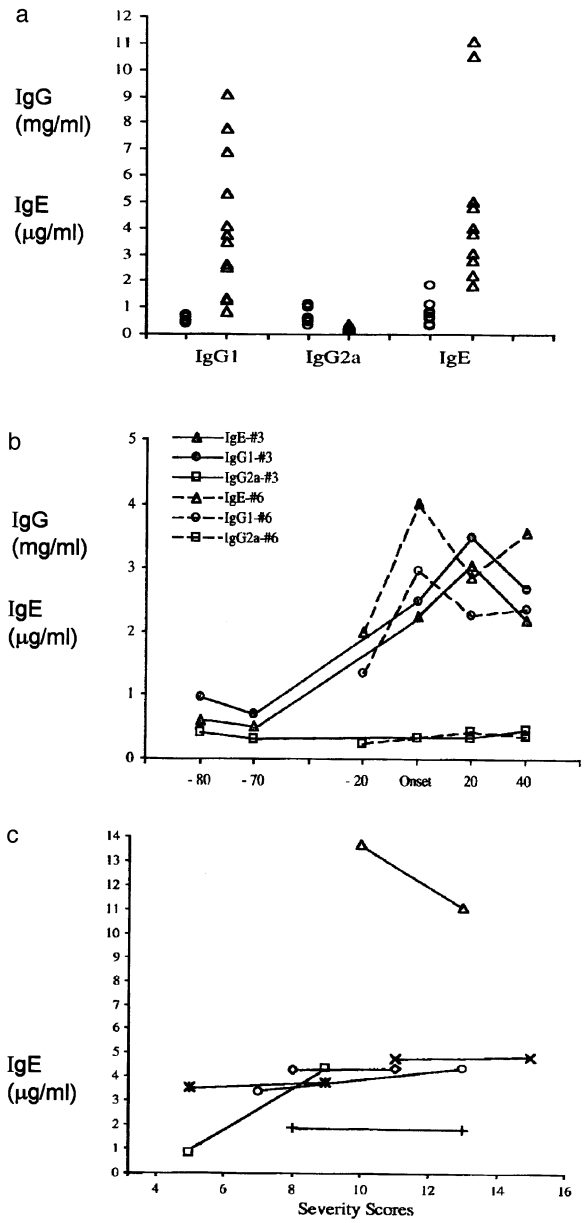

Figure 4. Total serum IgE and IgG1 concentrations in the affected IL-4 Tg mice are elevated and coincide with the onset and early progression of inflammatory skin lesions. In (*a*), total serum levels of IgE, IgG1, and IgG2a were examined for the affected Tg mice (Δ, $N = 12$) and the non-Tg littermates (○, $N = 8$) at one time point. In (*b*), total serum levels of IgE (Δ), IgG1 (○), and IgG2a (□) in relation to the onset of skin disease are illustrated ($N = 2$). The numbers on the horizontal axis (not in exact scale) marked by negative (–) signs represent the days before the onset of skin disease. #3 indicates Tg mouse #3, #6 indicates Tg mouse #6. In (*c*), total serum levels of IgE in relation to the progression of skin disease (as indicated by severity scores) in seven affected Tg mice are illustrated.

Prominent epidermal and dermal infiltrate of T cells characterized the early inflammatory lesions Immunopathology studies revealed that in the inflammatory lesions infiltrating CD3+ T cells were observed in the epidermis and dermis. In chronic inflammatory lesions, CD3+ T cells were observed in similar amount, but only in the dermis. Rare CD3+ T cells were observed in the non-Tg mice dermis. In the lesional skin, both CD4+ and CD8+ T cells were observed in approximately the same quantity (data not shown).

The elevation of Th2-type immunoglobulins characterizes the serology of Tg mice and correlates with the onset and early progression of skin disease ELISA assays determined that at one time point the total serum IgE and IgG1 levels in most affected Tg mice were highly elevated compared to non-Tg littermates (**Fig 4*a***). By contrast, the total serum IgG2a levels in the Tg mice were slightly reduced. The onset of the skin disease was associated with a concurrent elevation of IgE and IgG1, but not IgG2a (**Fig 4*b***). Moreover, the total serum IgE remained at high levels during further disease progression (**Fig 4*c***). The elevated total serum IgE levels in our IL-4 Tg mice resemble those observed in human AD patients (Leung *et al*, 1999) and in NC/Nga mice with an AD-like skin lesion (Matsuda *et al*, 1997). Furthermore, canine AD is also characterized by an elevation of antigen-specific IgE (Willemse, 1986).

Serum IL-4 levels were undetectable in Tg mice Neither the Tg mice nor the non-Tg littermates had detectable serum levels of IL-4.

DISCUSSION

Together, the clinical manifestation, the pathologic and immunopathologic findings, the microbiologic data, and the serum total IgE levels in our mouse disease model fulfill the current clinical diagnostic criteria of AD for human patients (Hanifin and Rajka, 1980). Our model fulfills three major diagnostic criteria, including pruritus, chronic dermatitis, and family history of atopy (dermatitis), and four minor diagnostic criteria including elevated serum IgE, staphylococcal skin infection, xerosis, and conjunctivitis. Our model also fulfills four major diagnostic features for canine AD, including pruritus, facial involvement, chronic dermatitis, and positive family history of atopy, and at least one minor diagnostic feature of staphylococcal skin infection (Willemse, 1986). We will term this disease model as a "pruritic inflammatory skin disease", pending further characterization on the detailed infiltrating inflammatory cell types, lesional cytokine profiles, and responses to immune modulators, in authenticating this model as a mouse counterpart of human AD.

AD has long been suspected to be a T-cell-mediated disease associated with a hyperactivated Th2 immune abnormality and a Th1 immune suppression. Bone marrow donors with atopic disease can transfer their disease to healthy recipients (Agosti *et al*, 1988). IL-4-producing T lymphocytes in AD are frequently observed in lesional skin and peripheral blood and can be spontaneously activated; this hyperactivated IL-4 production may be due to an interaction of altered nuclear protein with the IL-4 promoter (Chan *et al*, 1996). This concept is further supported by the observation that AD is improved by interferon γ, a Th1 cytokine (Leung *et al*, 1999). The role of Th1 lymphocytes in the pathogenesis of AD remains unresolved, however (Grewe *et al*, 1994; Hamid *et al*, 1994). Our model of pruritic inflammatory skin disease suggests that it is initiated by a Th2 cytokine IL-4 and provides the opportunity to delineate the role of Th1 cytokines in the chronic disease phase.

The inflammatory skin disease in our model primarily occurred in poorly haired skin (**Table I**), similar to the findings observed in canine AD and a spontaneous mouse AD-like skin disease (Willemse, 1986; Matsuda *et al*, 1997). In human patients, AD primarily involved infants' face, scalp (relatively hairless at that time), and extensor surfaces, but sparing the diaper area (which is protected by the diaper from contacting environmental factors) (Leung *et al*, 1999), and primarily affected adults' face, antecubital fossae, and propiteal fossae (Leung *et al*, 1999). Presumably, hairless areas have a greater exposure to environmental factors, which may play roles in the initiation of this inflammatory skin disease and AD and may explain the delayed onset of skin lesions in our model.

Histopathologically, actively degranulating mast cells were present in the early lesions of our model and these mast cells, though less active in degranulation, persist in the chronic lesions (**Fig 3**). The abundance of serum IgE in our mouse model could be

the source for the mast cell degranulation. Staphylococcal toxins, by augmenting specific IgE responses, could trigger mast cell degranulation (Hofer *et al*, 1999; Leung *et al*, 1999). Through the known actions of the inflammatory mediators in their granules such as histamines, prostaglandins, leukotrienes, and Th2 cytokines, the activated mast cells may participate in both the initiation and maintenance of inflammatory lesions. The absence of eosinophils in the early skin lesions and the moderate presence of eosinophils in the chronic skin lesions in our mouse model suggest that eosinophils do not have a significant role in the initiation of this inflammatory disease but may have a role in the maintenance of the disease. Previously, a minor skin inflammation, blepharitis, has been reported in mice transgenic for IL-4 under the control of nonskin-specific promoter (Tepper *et al*, 1990). Histologically, these skin lesions contain dense dermal infiltrates of mononuclear cells, mast cells, and eosinophils (Tepper *et al*, 1990), similar to those observed in the skin lesions of our skin-specific IL-4 Tg mice. Serologically, these IL-4 Tg mice also had elevated total serum IgE (Tepper *et al*, 1990), as observed in our IL-4 Tg mice. The reason that these IL-4 Tg mice contained in a pathogen-free facility did not develop severe inflammatory skin lesions *de novo* is not clear. One possible explanation is that although the IL-4 expressed through attenuated immunoglobulin promoter/enhancer altered the T cell population towards a biased Th2 subtype, the limited exposure of T cells to skin pathogens and perhaps also to allergens reduced the other factors essential for the development of severe skin lesions. Placing our skin-specific IL-4 Tg mice in both conventional and pathogen-free facilities and observing the frequency and severity of their skin lesion development may help to answer this question.

Four signal pathways involved in the initiation of Th2 immune responses have been identified, including IL-4, T cell receptor ligation, IL-12Rβ2 modulation, and IL-13 (Kopf *et al*, 1993; MacKenzie *et al*, 1999). IL-4 and IL-13 are essential for the synthesis of Th2-activated immunoglobulins IgG1 and IgE (MacKenzie *et al*, 1999). Molecularly, IL-4 controls IgE synthesis by providing the first signal to the B cell's IL-4 receptor, in conjunction with the second signal of interaction of CD40 and CD40L, triggering the isotype switching (Oettgen and Geha, 1999). In our model, this Th2 enhancement occurs not only in an organ-specific manner (evident by the development of pruritic inflammatory skin disease, an organ-specific immune-mediated disease), but also in a systemic manner (evident by the elevation of total serum IgE and IgG1, apparently through a local expression of IL-4) (**Fig 4**). This locally expressed IL-4 in the skin seems to be essential for restricting this disease to the skin, as multiple-organ expression of IL-4 induced systemic immune disorders (Erb *et al*, 1997). The concurrent elevation of total serum IgE and IgG1 and the onset of skin lesions in our model suggest that IgE and IgG1 may be essential for the initiation of the skin lesions. Moreover, the total serum IgE in the affected mice remained at high levels during disease progression suggesting a role of IgE in the maintenance of the disease. As not all affected mice have high titers of total serum IgE, antigen-specific IgE may be a more specific assay in determining the role of IgE in the pathogenesis of skin lesions.

Close to half of the Tg mice affected with skin inflammation developed bacterial pyoderma, whereas none of the Tg mice unaffected with skin inflammation developed any infection. The cause-and-effect relationship between the cytokines and infection in our model is not yet established. One hypothesis is that the pruritus associated with the skin lesions leads to scratching and excoriation, damaging the skin natural barrier and therefore opening the door for bacterial infection. Supporting this hypothesis is the observation that only those Tg mice affected with skin lesions developed bacterial infection. The occurrence of bacterial pyoderma does not correlate with the extent of skin lesions, however (**Table I**). Another hypothesis is that IL-4 expressed in the skin downregulates the skin immune system in defense against a certain type of pathogens, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, in such a way that even minor damage of the skin barrier (as a result of a scratch) will allow severe infection to occur. Observations from the studies of other skin infections lend some support to this hypothesis (Yamamura *et al*, 1991; Heinzel and Rerko, 1999). The presence of Th2 cytokine alone, however, does not seem to account for the staphylococcal infection, as the infection occurred only in those Tg mice affected with skin inflammation. The availability of this new experimental mouse model of pruritic inflammatory skin disease may facilitate the investigation of the relationship between Th2 cytokine and skin immunology in general and that between Th2 cytokine and immune defense against infections in particular.

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